Solenopsis invicta captured in a baited trap near Shell Mountain Bat Cave, Fort Hood, Texas Photo by Steve Taylor, 8 August 2001.

INVESTIGATION OF THE POTENTIAL FOR RED IMPORTED FIRE ANT (*SOLENOPSIS INVICTA*) IMPACTS ON RARE KARST INVERTEBRATES AT FORT HOOD, TEXAS: LITERATURE SURVEY AND STUDY DESIGN

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Caves are home to a variety of rare and unique invertebrates. Fort Hood (Bell and Coryell counties, Texas) cave communities include 19 karst[1] invertebrates of special concern (Table 1, Reddell 2001). Among these primarily cave-limited species are some taxa known only from Fort Hood. The Fort Hood karst invertebrate communities are threatened by the invasion of the red imported fire ant (RIFA), ***Solenopsis invicta*** Buren (Hymenoptera: Formicidae: Myrmicinae). This report outlines a proposed study design for examining RIFA impacts on karst invertebrates at Fort Hood.

Table 1. Karst species of concern at Fort Hood, Texas (Reddell 2001).

Amphipods [aquatic]	
	Stygobromus bifurcates
	Stygobromus russelli
Isopod [aquatic]	
	Caecidotea reddelli
Spiders	
	Cicurina caliga
	Cicurina coryelli
	Cicurina hoodensis
	Cicurina mixmaster
	Neoleptoneta n. sp.
	Neoleptoneta paraconcinna
Pseudoscorpion	
	Tartarocreagris hoodensis
Harvestman	
	Texella n. sp.
Millipede	
	Speodesmus n. sp.
Springtail	
	Lepidocyrtus dubius
Bristletail	
	Texoreddellia n. sp.
Beetles	
	Rhadine reyesi
	Batrisodes n. sp.
	Batrisodes feminiclypeus
	Batrisodes gravesi
	Batrisodes wartoni
Salamander	
	Plethodon n. sp.

Energy Flow in Caves

Caves are typically low-energy environments (Poulson and White 1969). Lacking sunlight as an energy source, the cave community is a decomposer community, relying on energy input in the form of organic matter brought into the caves by organisms, and organic matter which falls or is washed into the caves. Major energy sources include 1) remains of accidentals, organisms that fall or wander into caves and are trapped (e.g., in pit entrances) or cannot find their way out; 2) leaf litter and other plant debris that falls, washes, or is carried (e.g., for rodent nests) into the caves; and 3) the feces of

[1] Karst = A terrane, generally underlain by limestone or dolomite, in which the topography is chiefly formed by the dissolving of rock, and which may be characterized by sinkholes, sinking streams, closed depressions, subterranean drainage, and caves (USEPA 1999).

trogloxenes[2] that forage above ground (e.g., cave crickets, some harvestmen, bats). Caves in the study area (East Range of Fort Hood) rarely have active cave streams that bring in significant organic debris as one might see in some caves of the midwestern or eastern United States, largely eliminating this mode of energy input from consideration. However, debris falling into caves forms a significant, but highly localized, accumulation of nutrients – often in the twilight zone below the cave entrance.

Figure 1. ***Rhadine reyesi*** Reddell and Cokendolpher (Carabidae) in Big Red Cave, Fort Hood. This troglobitic beetle preys on the eggs of the cave crickets, and is one of the 'species of concern' listed by Reddell (2001). Photo by Steve Taylor and Vanessa Block, 3 September 2001.

Cave Organisms

Energy limitations discussed above, and the unique, isolated and stable environment[3] of these caves (Poulson and White 1969) have led to the presence of relatively simple cave communities. In central Texas caves, including those of Fort Hood, these communities are comprised of a variety of uncommon, troglobitic[4] invertebrates (e.g., Figures 1,2), along with some troglophiles[5] and trogloxenes (Reddell 2001). As is the case in a number of other cave regions, some species are endemic to one or very few caves in central Texas and are found nowhere else in the world[6]. Compared to their epigean (above ground) relatives, these

[2] trogloxene – a species that does not normally feed in caves but which may enter them (Humphreys 2000).
[3] Temperatures in the dark zone of Fort Hood caves is about 69 ºF year round, and the humidity is high and stable.
[4] troglobite – a species which does not exist outside of caves, the upper hypogean zone, or superficial underground compartments (after Humphreys 2000).
[5] troglophile – a species which is able to complete its life cycle in caves, but also can do so in epigean environments (roughly following Humphreys 2000).
[6] At Fort Hood, for example, *Cicurina mixmaster*, an endemic troglobitic spider, is known only from two caves.

organisms exhibit a host of characteristics typical of cave-limited species (Culver 1992, Howarth 1983). Among these characteristics are: reduced metabolic rates, slower movements, elongate (attenuated) appendages, tactile structures and long sensory hairs, reduced or absent eyes, reduction or loss of pigment in the integument, long life cycles, fewer and larger eggs, and enhanced chemosensory abilities. Due to resource limitation, populations are typically sparse.

Given the above factors, it is not surprising that these organisms are infrequently observed by the casual cave visitor. Even experts may need several visits to a cave before detecting some of the rarer species, which are often predators. Because these taxa would be rare and infrequently observed, a study design dependant on encountering troglobites with sufficient regularity to obtain statistically meaningful sample sizes is not prudent.

Figure 2. A cave spider of the genus ***Cicurina*** (upper right) in Big Red Cave. Note several foraging fire ants in the foreground (left). Photo by Steve Taylor and Jean Krejca, 9 Augsut 2001.

The available data on cave systems (e.g., Wilkens et al. 2000) clearly demonstrates the dependency of troglobites on scarce resources obtained from epigean habitats. That is, the rare karst invertebrates depend upon a natural influx of nutrients in the form of organic material –

fecal material from major trogloxenes (e.g., cave crickets), leaf litter, and animals (both dead and alive).

In particular, cave crickets are an important energy source for cave communities. Each night during the active seasons, large numbers of these crickets forage in the vegetation outside the cave entrances, returning to spend their days in the sanctuary of the caves. ***Ceuthophilus*** spp. (cave crickets, Orthoptera: Rhaphidophoridae) are opportunistic omnivores. For example, in New Mexico caves Campbell (1976) noted both animal and plant material in the stomachs of ***Ceuthophilus conicaudus***, and Cokendolpher et al. (2001) collected ***Ceuthophilus carlsbadensis*** and ***Ceuthophilus longipes*** at a variety of bait types (jelly, tuna, and rancid liver), with bait preferences varying seasonally. Elliott (1992) made observations on foraging by ***Ceuthophilus secretus*** and an undescribed but closely related species (in Travis and Williamson counties, Texas), noting they "were mostly seen on foliage, dead leaves, lichens on sticks, and grass, but they were not chewing although they used their palpi to probe the substrate." Elliott (1992) also observed a cave cricket with a dead RIFA in its mandibles. According to Reddell (personal communication, August 2001), adults of the two ***Ceuthophilus*** species that Elliott worked with in Travis and Williamson counties (Elliott 1992) seem to be dominant at different times of year. At Fort Hood, only one of these two species, ***Ceuthophilus secretus*** (Figure 3), is present. ***Ceuthophilus cunicularis***, a species rarely observed foraging outside of caves, also is present in Fort Hood caves, but is generally much less abundant than ***Ceuthophilus secretus***.

The health of the epigean community is important in the life cycles of surface-foraging cave crickets. Because these crickets are a dominant feature of the biota of Fort Hood caves, they contribute greatly to the energy input into the caves, and thus are critical to the well being of the rarer, troglobitic invertebrates. Reddell and Cokendolpher (2001b) note that "Probably the greatest ultimate impact on the ecology of caves by fire ant predation is the reduction of cave cricket populations."

Figure 3. ***Ceuthophilus secretus*** in Big Red Cave. Photo by Steve Taylor, 3 September 2001.

The Red Imported Fire Ant, an introduced species

Solenopsis invicta, native to South America, entered the United States in the early 1900's in Mobile, Alabama, and has since spread across the southeastern United States (Vinson 1997, Taber 2000) and into several states further west (Callcott and Collins 1996). The species has had a profound effect on community ecology (Vinson 1991, 1994; Wojcik et al. 2001), and its control has been problematic (Banks 1990, Jouvenaz 1990, Lofgren 1986, Lofgren et al. 1975, Williams 1994). The red imported fire ant is well established in Texas (Cokendolpher and Phillips 1989, Hung and Vinson 1978, Porter et al. 1991), where its range now encompasses Fort Hood (Reddell 2001, Reddell and Cokendolpher 2001b). Taber (2000) suggests the northern extent of the range of RIFA is likely to be limited by low temperatures, whereas western limits of distribution are likely to be restricted by availability of water.

Association of *Solenopsis invicata* with Caves in Texas

Figures in Elliott (1992) show that caves he examined had multiple RIFA mounds within 15 m of the cave entrances. Elliott's (1992) data indicate that RIFA are more likely to use caves, or at least cave entrances, during the hottest, driest part of the year (July-August). Taber (2000) found that "during dry spells in Central Texas it can be difficult to find even a single mound," and this supports Elliott's observations. Preliminary field work at Fort Hood in August 2001, conducted during the preparation of this proposed study design, revealed few fire ant mounds showing activity even with persistent disturbance by probing – but foraging RIFA were common in wooded, shady areas despite the absence of mound activity. By September, with moderated temperatures following a week of intermittent rain, RIFA mound activity and foraging in all epigean habitats were much more evident (SJT, unpubl. data, 2001; see photo on cover of this report).

Elliott (1992) summarized earlier observations of RIFA preying upon invertebrates in caves. Prey included earthworms, a scorpion, a pseudoscorpion, several millipede taxa, springtails, bristletails, a ***Plethodon*** salamander, and both live and dead cave crickets. Most of these organisms are found on cave floors, but Elliott (1992) cites observations by Reddell of predation upon millipedes and cave crickets on the ceilings of caves. Reddell and Cokendolpher (2001b) observed that "During the most severe infestations the entire floor and much of the walls of the cave are carpeted with ants." Elliott (1992) also described RIFA foraging trails extending well into the dark zone of at least one cave, and he noted ant abundance generally decreased with increasing depth and distance into the caves. Elliott (1992) also observed RIFA in the dark zones of caves may seem more sluggish than those at cave entrances or in epigean habitats – this appears to be related to temperature (see Rissing 1982, Marsh 1985).

Soil moisture, temperature, humidity, and time of day all are known to affect the foraging activity of ants (Talbot 1943). Porter and Tschinkel (1987), found relationships between temperature and foraging activity in RIFA, but humidity and soil moisture were not important. Potts et al. (1984) found that workers of RIFA alone did not show humidity preferences, but when tending their brood they exhibited a strong preference for high humidity. Temperature preferences of RIFA also appear to vary seasonally and under differing humidities (Cokendolpher and Franke 1985). Korzukhin et al. (2001) conclude that temperature is the most important environmental parameter influencing activity and metabolism of RIFA colonies, and Porter and Tschinkel (1987) found soil temperature at 2 cm depth was a better predictor of foraging rates than any other parameter in their study. The fire ants are known to forage at night and in the daytime during favorable times of year (e.g., Porter and Tschinkel 1987), and at Fort Hood I have observed (September 2001) foraging trails of RIFA extending into Big Red Cave (Figure 4) actively in use by the ants both in the daytime and at night.

Fire ants are aggressive and opportunistic omnivores that are able to capitalize on localized resources (Taber 2000, Wilson and Oliver 1969, Wojcik et al. 2001). While it is already known that these ants forage in caves, most evidence is based on collection records (Reddell 2001, Reddell and Cokendolpher 2001b) and anecdotal observations,

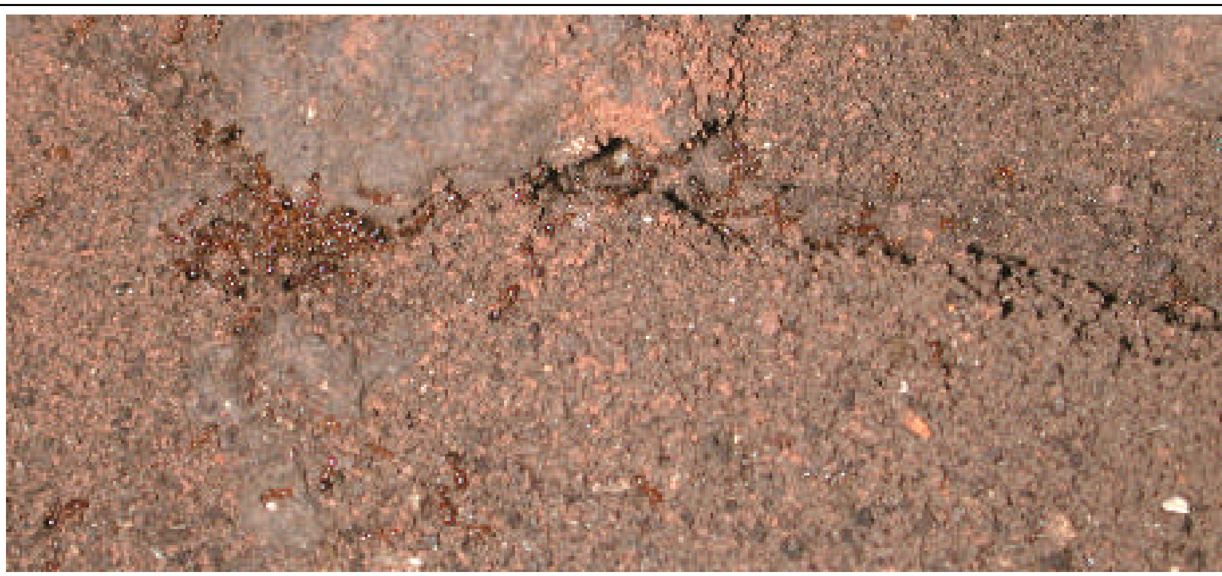

Figure 4. A very active foraging tunnel of ***Solenopsis invicta*** at the bottom of the entrance drop of Big Red Cave. Presence of such large numbers of these ants this far (35 feet below the surface) into the cave is an indication of a serious fire ant infestation. Photo by Steve Taylor and Jean Krejca, 9 August 2001.

or has focused on identification of potential control methods (e.g., Elliott 1992). Various reports to agencies by Elliott (cited in Elliott [1992]) documented sometimes heavy cave use by RIFA in the Austin, Texas area, but the fairly extensive list of caves at Fort Hood where RIFA have been recorded (Reddell 2001, Reddell and Cokendolpher 2001b) generally lack quantitative density measures. A major goal of the study proposed here is to quantify the ***extent to which the fire ants utilize caves*** at Ft. Hood.

The use of Ft. Hood caves by RIFA, documented by Reddell and others (Reddell 2001, Reddell and Cokendolpher 2001b), makes it clear that RIFA ***do*** have an impact on rare karst invertebrates. This is based on the biology of cave invertebrates (briefly outlined above), the massive literature documenting aggressive dominance of RIFA in the communities it invades (Wojcik et al. 2001), and results of studies of RIFA/karst invertebrate interactions reported by Elliott (1992) and various other records of RIFA collections from caves reported in Reddell and Cokendolpher (2001b). RIFA infestations typically result in a simplification of the biotic community being dominated by the ants (Morris and Steigman 1993, Porter and Savignano 1990, Jusion-Atresino and Phillips 1994, Wojcik et al. 2001).

Direct and Indirect Impacts of RIFA on karst invertebrates

RIFA impacts on karst invertebrates can be broken into two broad categories: direct impacts and indirect impacts (e.g., Strauss 1991).

Direct impacts on the species of concern (Table 1) can be examined by studying the life histories and biology of the cave organisms, and by gauging the intensity of fire ant utilization of the cave environment. Many of the cave-adapted species are rare, for reasons previously outlined. Typically, no more than a few individuals of these species are observed during a cave visit, and this is especially true of the predatory taxa. Consequently, 1) it is unlikely that statistically meaningful numbers can be generated by monitoring these rare invertebrates; and 2) studying the biology of rare troglobites could have negative impacts on their populations (through over-collection, disturbance, etc.).

Therefore, studies of the biology (or even life histories) of these rare taxa will not be the focus of the study proposed herein – work with these taxa will be limited to visual censusing.

Presence of RIFA in the caves implies direct impacts – competition with, and predation upon, the karst invertebrates (Figure 5). Furthermore, RIFA predation within caves upon ***Ceuthophilus*** nymphs constitutes another direct impact, and has been described as "very heavy" (Reddell and Cokendolpher 2001b), further threatening the health of cave cricket populations. The extent of these various direct impacts can be estimated by the foraging intensity of RIFA in the caves and by examining the seasonality of their in-cave activity. Thus, in-cave ant activity (as measured by numbers of RIFA individuals recruited to baits) is indicative of RIFA impacts upon the rare karst invertebrates. Methods outlined below are designed to examine direct impacts by studying foraging activity of RIFA in the caves.

Indirect, or secondary, impacts include any effects RIFA may have on the flow of energy into the cave system. The presence of RIFA outside of caves, where the cave cricket ***Ceuthophilus secretus*** forages, appears to constitute such an impact. ***Ceuthophilus secretus*** forage outside of caves at night, returning to the caves in the daytime. In their diurnal habitats, the feces, bodies of dead crickets, and cricket eggs deposited in soft cave-floor sediments, collectively constitute major contributions to the energy input into the caves. This energy is critical to the long-term health of the karst invertebrate species of concern. If RIFA monopolize resources above ground, out-compete ***Ceuthophilus secretus*** for energy-rich foods, or prey upon foraging ***Ceuthophilus secretus*** during their nighttime excursions (Figure 6), then there are important indirect effects of RIFA upon the rare, cave-limited karst invertebrates. Assessing the extent of these indirect impacts requires that we are to some degree able to quantify the size of the foraging range of ***Ceuthophilus secretus*** populations in the study area.

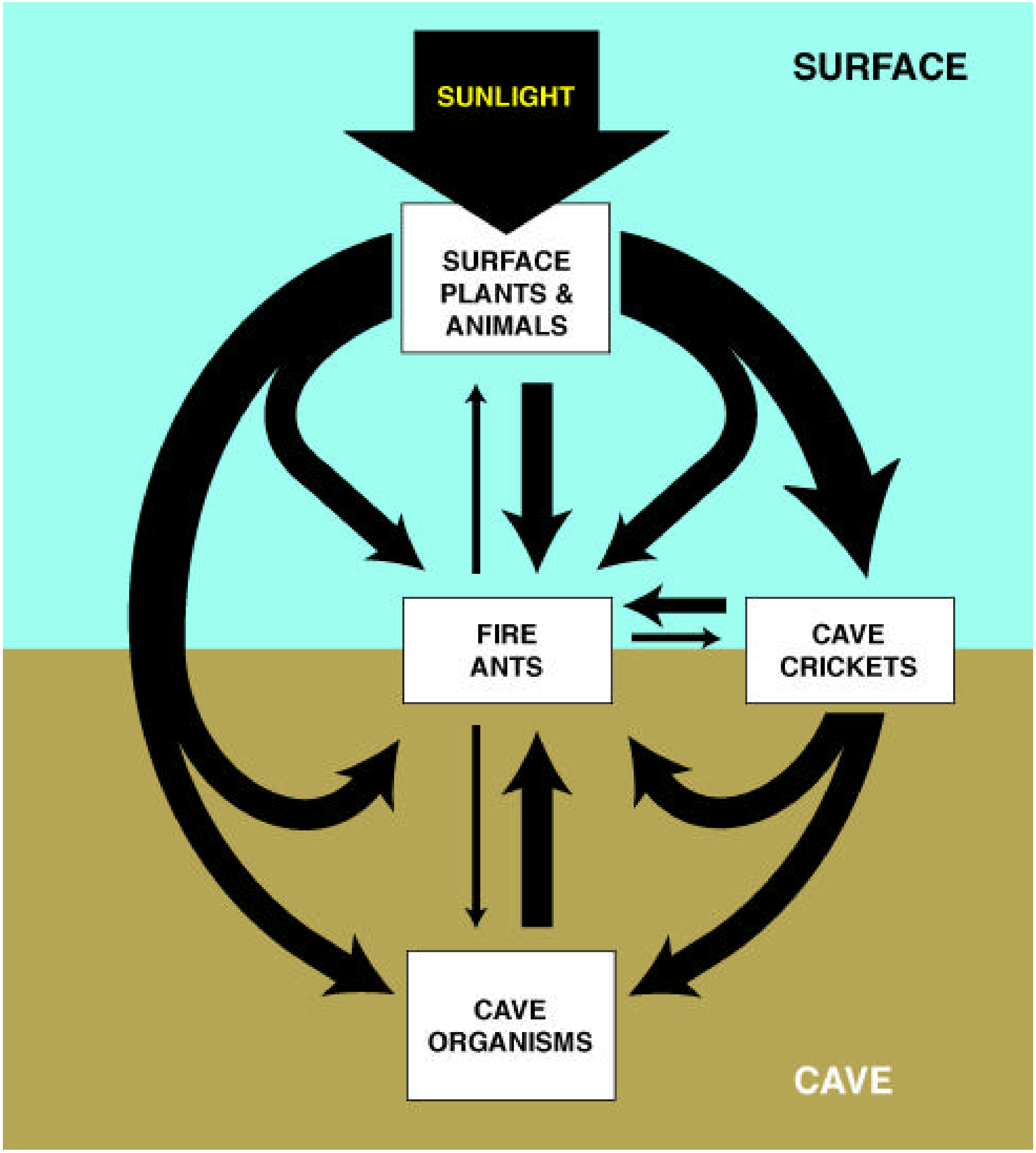

Figure 5. Simplified energy relationships of a hypothetical RIFA-compromised cave system. Some portion of the energy that would go to crickets (and other trogloxenes, e.g., harvestmen, mice, snakes, salamanders, bats, etc.) is taken by fire ants. Not shown are other energy sources that might feed into the cave community, for example, connections with interstitial spaces through which cave organisms or edaphobites might enter the caves. Roots of trees and other plants likely extend down to the cave, both providing some nutrients and extracting some water from the system.

Figure 6. ***Solenopsis invicta*** foraging on the remains of a cave cricket, ***Ceuthophilus secretus***, just outside of the entrance of Big Red Cave, Fort Hood, Texas. Worker size variation (majors, medias, and minors) is obvious in the photo. These adult ants do not feed on solid material – larger food particles are returned to the colony for digestion by the oldest larvae. It is unclear whether this photograph documents an instance of scavenging or one of predation. Photo by Steve Taylor, 9 August 2001.

From a management perspective, it is important to quantify three things: 1) the extent to which RIFA forage inside of the caves; 2) the extent to which RIFA forage above ground near caves; and 3) the foraging range of ***Ceuthophilus secretus*** outside of the caves. Effective management of karst resources – ensuring the long-term survival of cave-limited invertebrates – requires maintaining a natural community above ground that encompasses the foraging range of ***Ceuthophilus secretus*** with a buffer to account for edge effects (Laurance and Yensen 1991). Such management should include both controlling fire ants within the area defined and limiting habitat disturbance.

The research question

The task of this report is to develop methodologies to address the following question:

Does the RIFA have an impact on rare and endangered karst invertebrates at Fort Hood?

As previously discussed, the answer to the above question is already known – the ants do have an impact. I have reworded the question here to facilitate proposing the study design which is presented in the following section of this report:

To what extent are RIFA a threat to karst invertebrate communities at Ft. Hood?

Specific questions addressed in the study design

To address the above research question, I propose examining several aspects of RIFA impacts on karst invertebrates through two more specific research questions:

1. To what extent do RIFA enter and forage in caves?
2. How much land area around a cave needs to have RIFA control measures implemented to protect the cave fauna?

The first question examines direct impacts of RIFA on the species of concern, while the second (through the study of ***Ceuthophilus secretus*** foraging) examines indirect impacts.

Expanding on the basic questions

Bold text in the list below refers to methods which will address the question. More detailed methods are outlined in the next section.

To what extent are RIFA a threat to karst invertebrate communities at Ft. Hood?

1. To what extent do RIFA enter and forage in caves?
    i. How far into caves do RIFA regularly forage? (**In-cave timed bait trapping, supplemental observations of RIFA from in-cave quadrat census**)
    ii. Are there seasonal patterns to RIFA foraging in the caves? (**comparison of in-cave timed bait trapping data across seasons**)
    iii. How do seasonal patterns of in-cave RIFA foraging relate to surface RIFA foraging activity and to fluctuations in temperature? (**comparison of in-cave timed bait trapping data and surface plot timed bait trapping across sample periods**)
    iv. To what extent does in-cave RIFA foraging vary among caves? (**comparison of in-cave timed bait trapping data among caves**)
    v. How is the distribution of cavernicoles in the caves related to the distribution of RIFA in the caves? (**In-cave quadrat visual censusing compared to in-cave timed bait trapping**)

2. How much land area around a cave needs to have RIFA control measures implemented to protect the cave fauna?
    i. How far away from cave entrances does ***Ceuthophilus secretus*** forage? **(Marking emerging crickets at cave entrance, recording location during foraging)**
    ii. Do RIFA affect foraging efficiency of ***Ceuthophilus secretus***? **(RIFA-excluded baits versus RIFA-allowed baits, video sessions)**

Methods

Timing of sampling

Bait trapping for RIFA and in-cave quadrat censusing will be carried out at each site on a bi-monthly basis (six times per year). Sampling will continue for a full year, and additional sample periods will be added as funds and time permit. Studies of epigean foraging by ***Ceuthophilus secretus*** will not be conducted when sample periods coincide with weather conditions that are unsuitable for cricket foraging activity (likely one or two winter sample periods). Seasonal variation in cricket foraging will not be examined because analysis of the available resources (time, money, personnel) suggest that this would not be feasible under the present circumstances. Cricket foraging studies may be conducted during some 'off months' between the bi-monthly in-cave studies and surface baiting for RIFA.

Selection of Caves

Study caves will be selected, in part, based on the number of species of concern present at the sites. Using data in Reddell (2001), I tallied the occurrence of 'taxa of concern' (Table 1) for each cave; caves harboring two or more of these species are listed in Table 2.

Table 2. Caves with two or more karst species of concern. Adapted from Reddell (2001).

Cave	Training Area	Cave Length (feet)	Cave Depth (feet)
Big Red Cave	2	353.00	63.20
Camp 6 Cave No. 1	6A	70.00	24.30
Chigiouxs Cave **	80	71.60	23.20
Fellers Cave	6A	48.00	35.00
Keyhole Cave	2	75.00	50.20
Mixmaster Cave	2	1020.85	35.00
Price Pit Cave	3	43.60	34.40
Keilman Cave **	3	3.00	2.00
Figure 8 Cave	6A	95.90	41.00
Nolan Creek Cave **	15A	115.00	10.00
Streak Cave	3	202.90	30.90
Talking Crows Cave	6A	68.20	19.30
Treasure Cave	6A	25.00	17.00
Bumelia Well Cave	6A	145.90	90.20
Triple J Cave	3	339.70	51.00
Lucky Rock Cave	6A	98.00	48.00
Peep in the Deep Cave	6A	75.00	20.00
Rocket River Cave System **	75	2 miles+	----
Tippit Cave **	75	300.00	55.00
Buchanan Cave	6A	158.30	65.00

** Caves excluded from study.

I excluded Chigiouxs Cave, the Rocket River Cave System, and Tippet Cave, because they are in the live fire area at Fort Hood and arranging for regular visits throughout the year would be problematic. Keilman Cave is excluded because its atypically small size would be difficult to incorporate into our study design, and the dimensions given (length and depth figures are from Nature Conservancy ArcView® files for Ft. Hood caves) suggest it might better be classified as a sinkhole than a cave. Finally, Nolan Creek Cave is excluded because two of the three species of concern present in that cave (the amphipod ***Stygobromus russelli*** and the aquatic isopod ***Caecidotea reddelli***) are aquatic, and I anticipate the results of this study will be more directly applicable to terrestrial taxa. Furthermore, Nolan Creek Cave is separated from the remaining caves by a relatively major surface drainage (Cowhouse Creek) – the remaining caves are found in a relatively well defined, and to some extent homogeneous, area in the eastern part of Fort Hood (Figure 7). This leaves a total of fifteen caves of varying length and depth (Table 2).

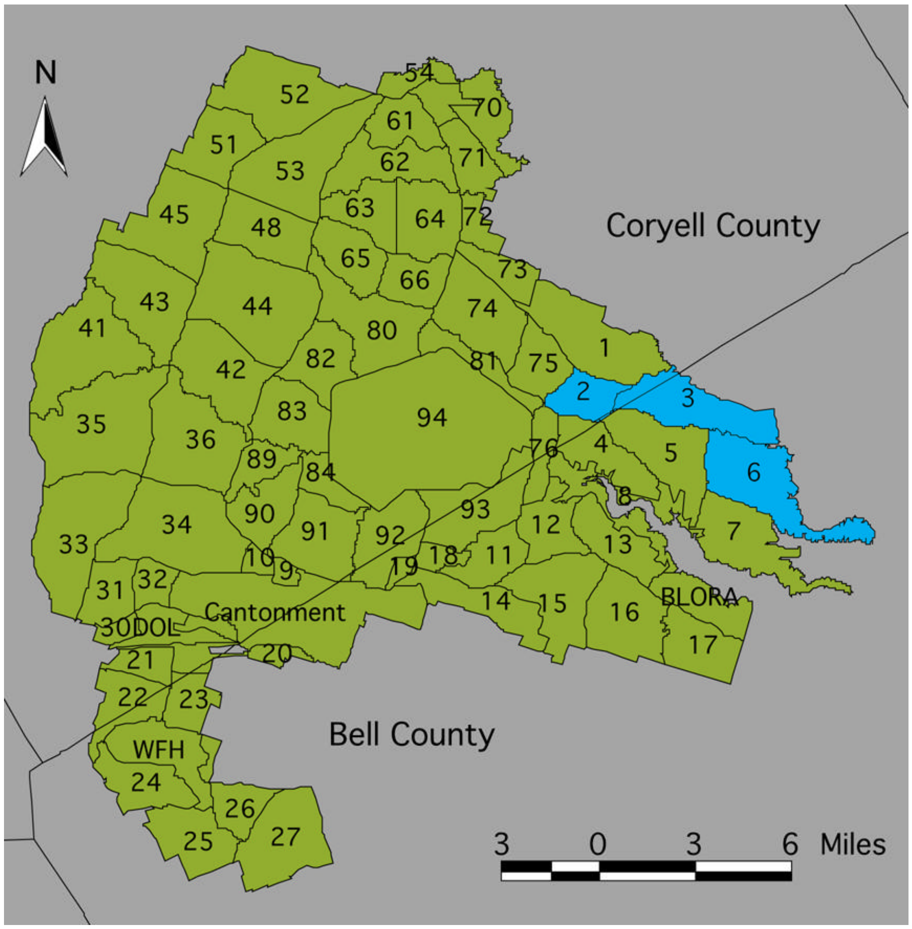

Figure 7. Fort Hood, Texas, with training areas shown. Study sites will be in training areas 2, 3, and 6 on the eastern side of the base.

From this list of caves, six caves initially will be selected for regular sampling. More caves will be sampled if feasible, less if six cannot be regularly sampled with available resources. The decision as to which caves will be used as study sites depends in part upon the number of karst species of concern found at each site, and upon site-specific factors (potential access problems or other unforeseen difficulties) which may not be discernable until visits to all potential field sites have taken place. Finally, field observations from preliminary trips suggest that there is more impact (soil disturbance) from military operations in training areas 2 and 3 than in 6A. Disturbance is thought to be an important factor (Sousa 1984) explaining RIFA distribution and abundance (Tschinkel 1986, 1998), and, therefore, an effort will be made to select sites with varying levels of disturbance.

Surface sample plot size

A consideration in choosing plot size is the foraging range for RIFA. RIFA construct foraging tunnels that may extend up to 30 m from their mound (Taber 2000). However, more typical home ranges for a colony are about 10-15 m in diameter or less (Wilson et al. 1971, Markin et al. 1975), and maximum territory area for a colony is around 100 m^2 (Korzukhin et al. 2001, Tschinkel et al. 1995). The size of the territory for monogyne[7] colonies can be determined from mound volume (Tschinkel et al. 1995), but this technique is not as accurate for polygyne[8] fire ants, whose populations can be twice the density of those of monogyne forms (Macom and Porter 1996). Figures in Elliott (1992) show numerous fire ant mounds within 15 meters of the entrances of caves. The foraging range of cave crickets is also a factor in determining plot size. Elliott (1992), working with ***Ceuthophilus secretus*** and a closely related, undescribed species, noted that "Cave crickets mostly feed within 5 or 10 m of the cave entrance, but large adults may travel 50 m or more." Based on Elliott's work (1992, 1994), it is thought that most cave crickets forage within 30 m of the entrances of caves (Reddell and Cokendolpher 2001b). This observation suggests a study plot 30 meters across would be sufficient to quantify RIFA impacts on the caves. Another factor affecting plot size choice is the "grain" of the epigean habitat. The distribution of wooded and grassy areas at some cave entrances is very patchy, and it is important to ensure that both are well represented when habitat types are variable. A final factor to be considered in determining plot size and number of sample points is to take into account the time and labor involved in setting up and regularly sampling plots. Given all of the above considerations, optimal plot size was determined to be a plot with 15 m radius, centered on the cave entrance. A square plot is practical way to facilitate placement of arrays of bait traps, and

[7] monogyne – colony characterized by the presence of only one egg-laying queen (Taber 2000).
[8] polygyne – colony characterized by the presence of two or more egg-laying queens (Taber 2000).

measures of vegetation, soils, etc., so actual plots will be square, 30 m long on each side, thus covering an area of 900 m^2, or 0.09 hectare (0.222 acre). Plots generally will be oriented with sides facing major magnetic compass bearings (N, S, E, W) to facilitate ease of presentation of data.

For each 30 x 30 m plot, a series of regularly spaced sampling points will be established. These grid points will be placed at 7.5 m intervals, resulting in a total of 25 points – 24 with the cave entrance excluded (Figure 8). Location of points will be established with the aid of compass and fiberglass tape. Points lying directly on RIFA mounds will be offset by 0.5 m, and the central point, overlying the entrance, will be offset 0.5 m as needed for some data collection (e.g., placement of bait trap).

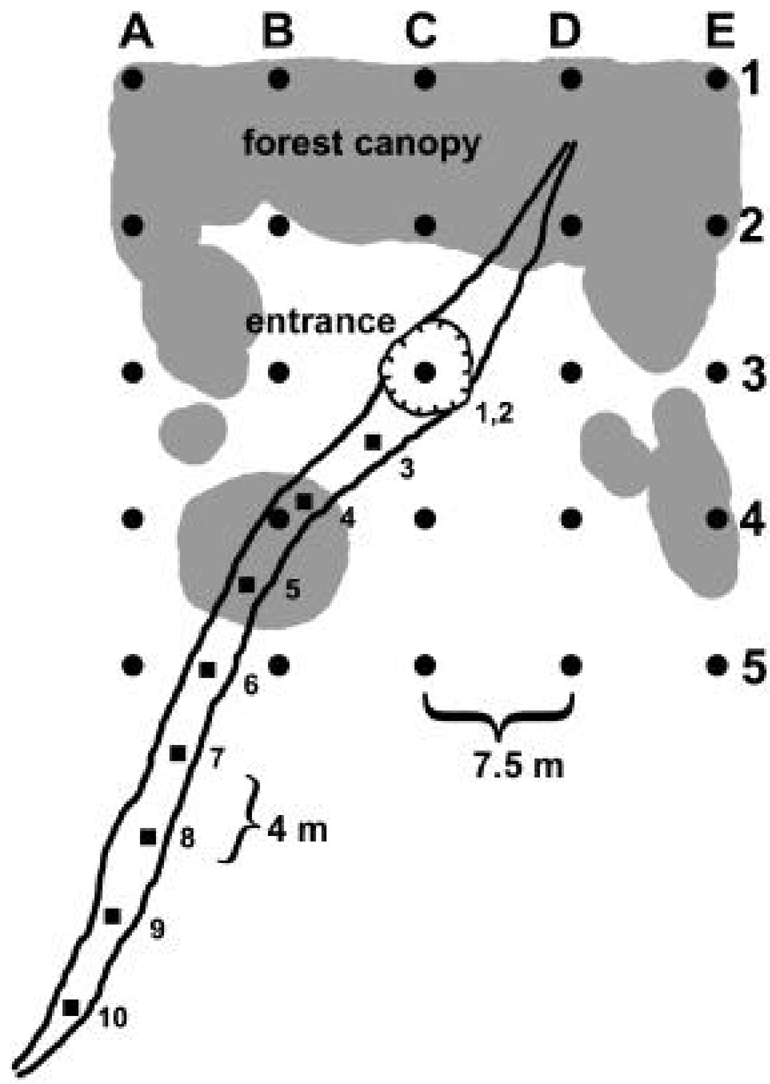

Figure 8. Generalized example of a hypothetical field plot showing surface sample point grid (circles) and in-cave sample transect (squares). Numbers and letters indicate sample point numbering system.

Figure 9. Ground cover quadrat at a surface station at Big Red Cave. Photo by Steve Taylor, 7 August 2001.

One-time sampling of Surface Plots

At each of the sampling points in each 30 x 30 m plot, a series of parameters will be measured on a one-time basis (ground cover, canopy cover, depth to impenetrable horizon) at 7.5 m intervals.

Ground Cover class (bare soil, rock, woody plants, grass, dead wood, leaf litter) will be tallied in 0.5 m^2 quadrats (0.701 x 0.701 m, Figure 9) based on digital images. The digital photo is taken at waist height (ca. 1 m), centered over the quadrat as much as is feasible. An array of 10 x 10 sample points will be overlaid on the digital photo, with the array stretched and distorted to account for the perspective view of the digital image

(Figures 10, 11). The nature of the substrate will be tallied for each point on the image. These numbers will be used to determine percent rock, soil, organic debris, wood vegetation, and herbaceous vegetation (Figures 12, 13).

Canopy Cover will be estimated using digital photographic methods. Photographic methods for canopy cover are similar to those for ground cover, except there is no physical quadrat. The area of the canopy sampled reflects the field of view of the digital camera, with a square sampled out of the middle of the image. A square array of points, extending to both edges of the short dimension of the image is overlaid on the image, for a total of 100 points arranged in a 10 x 10 matrix. The ends on the long axis of the image are not included in the analysis (Figures 14, 15). Each point on an image is scored only as open or canopy (Figures 16, 17). Four images will be recorded for each grid point – each with the researcher standing at the grid point and facing a different compass point for each image. The average score of the four images will constitute the estimate of percent canopy cover for the grid point. Canopy cover is important in explaining seasonal variations in RIFA foraging activity (e.g., Fleetwood et al. 1984).

Depth to impenetrable horizon will be determined at each point using a tile probe, with penetration measured with a ruler.

Bi-Monthly Sampling of Surface Plots

Soil temperature at each sampling point and RIFA foraging intensity (timed bait trapping) are data that will be collected on a bi-monthly basis. This sampling will be conducted seasonally to obtain data during the peak hot/dry season (July/August) and also in less extreme conditions. Using the array of points previously established in each 30 x 30 m surface plot, timed bait trapping of RIFA will be carried out to estimate RIFA foraging intensity during each bi-monthly sampling period. At each sample point in each plot, timed RIFA baiting will be accompanied by a measurement of soil temperature (at 2 cm depth, less if impenetrable to that depth) using a digital temperature probe. Bait traps consist of 15 ml polystyrene centrifuge tubes on the ground (Figure 18) with a 15x15 cm

Figure 10. Ground cover quadrat photo at Big Red Cave. The images that follow show how the ground cover classes are tallied.

Figure 11. The same image with a grid overlaid. There are 100 intersections in the grid (edges are not counted), which has been distorted in Photoshop to account for the perspective view of the image.

Figure 12. Colored dots, corresponding to different cover classes (grass, bedrock, etc.) are added to a third layer in the Photoshop image file.

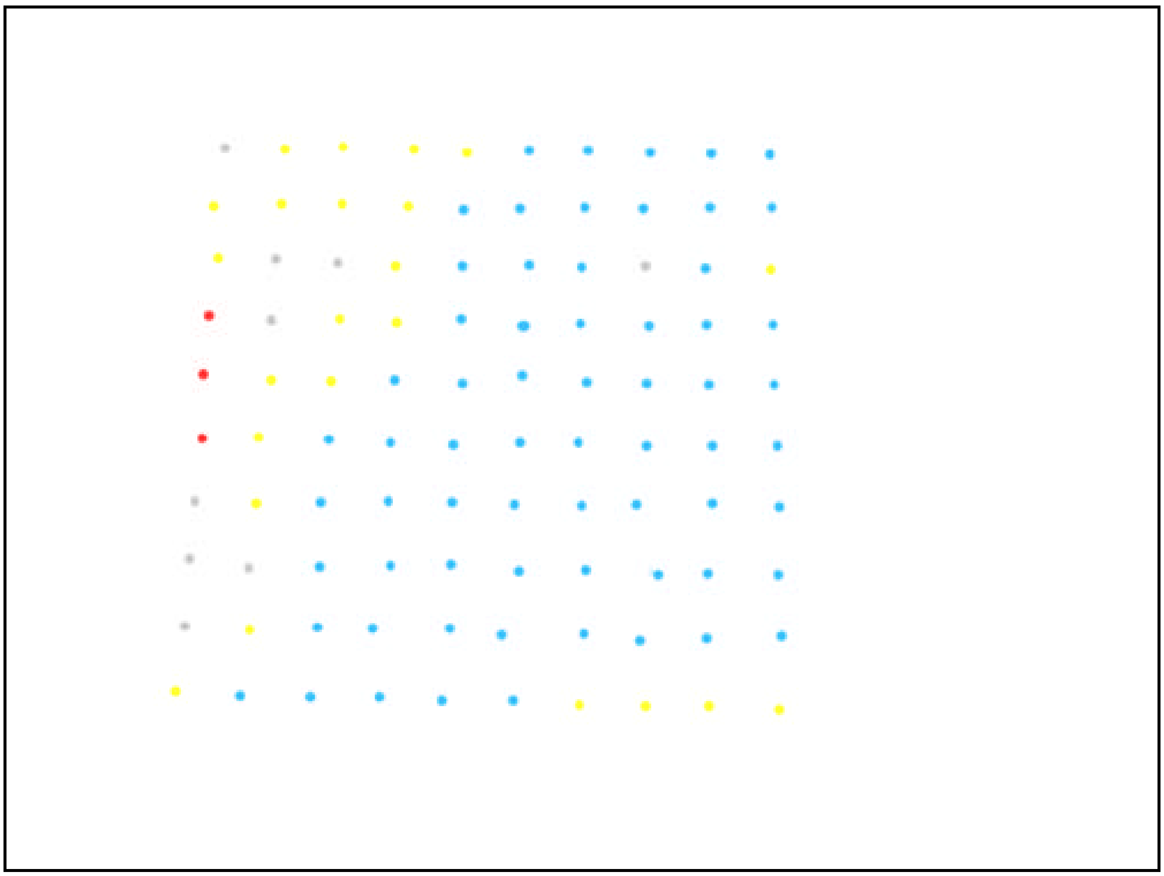

Figure 13. All layers except the colored dots (representing ground cover classes) have been turned off in Photoshop, and the dots are counted, by color, to give an estimate of percent ground cover for each cover class.

Figure 14. Canopy cover at Big Red Cave, station B1. The digital camera image above was imported into Photoshop, and a method similar to that outlined above for ground cover was used to tally canopy, as shown in the following images.

Figure 15. An array of 100 dots is placed on another layer in Photoshop. Note the small red dots in the corners that facilitate alignment of the new layer, so that sampling is always done in the center of the image.

Figure 16. The dots centered over canopy are marked on a third layer in Photoshop.

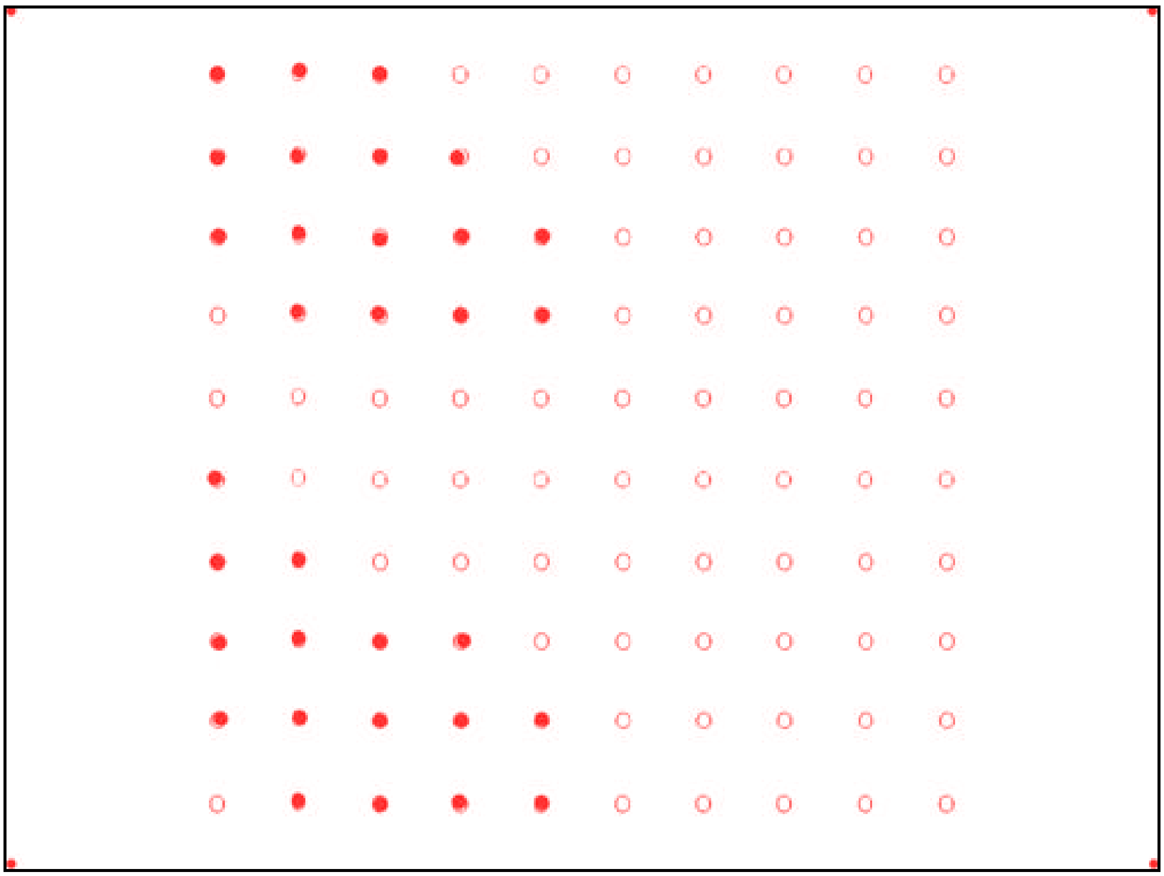

Figure 17. The original image is turned off in Photoshop, and the number of marked dots is tallied to estimate percent canopy cover.

square of cardboard placed over them for shade and a wire flag to facilitate relocation. This procedure is similar to that used by Porter et al. (1992) and Porter and Tschinkel (1987). Traps will be baited with Vienna Sausage, sliced into 0.5 cm thick disks, which are cut into four equal quarters, in a manner similar to that used by Porter et al. (1992). Bait traps will be left in place for 20 minutes, the time interval utilized by Porter et al. (1992). Porter and Tschinkel (1987) used a 30 minute sampling period, but concerns

Figure 18. A bait trap (cardboard cover has been removed) with ***Solenopsis invicta*** foraging upon Vienna Sausage (bait) near Shell Mountian Bat Cave. Photo by Steve Taylor, 8 August 2001.

about recruiting large numbers of ants into caves led me to choose the reduced sampling period for the proposed study. After the 20 minute sample period, collected traps will be immediately capped and placed in a bag with a small amount of ethanol. Ants from each trap later will be counted as a measure of foraging intensity. Porter and Tschinkel (1987) discuss several limitations of this type of baiting, including 1) concentrated and attractive bait may not be representative of natural foraging; 2) shading traps biases sampling towards foraging intensity away from direct sunlight; 3) temperature is thought to influence recruitment rates, so the sampling period is not independent of temperature; 4) available surface area of the bait, and the size of the tube, limit the number of foraging ants when large numbers of ants are present. Despite the above relatively minor concerns, this sampling method has been widely and successfully used (Bestelmeyer et al. 2000).

Data collected on a one-time basis, In-cave sampling transect

At each cave, a semi-permanent sampling transect with numbered stations at 4 meter intervals will be established using a fiberglass survey tape. Stations will begin just inside the entrance of the cave, and will continue for 10 stations (40 meters) in all caves where reasonably accessible passage extends at least that far. In smaller caves, fewer stations will be placed as the morphology of the study site dictates. Stations will be established on the floor of the human-traversable part of the passage in horizontal passages, offset to one (randomly selected) side of the passage to minimize disturbance by passing researchers. In vertical passages, stations will be placed on the nearest convenient ledge. To limit subjectivity in station placement, the vertical passage stations will be established by selecting a random major compass bearing (using a random numbers table: N, S, E, W) and then searching for a ledge within 0.3 m of that position. Failing that, the next random compass point will be selected, and the process is repeated until a suitable ledge is found. If no ledges are available within 0.3 m of the surveyed station position, a bolt will be set in the wall of the cave (using standard vertical caving techniques [Smith and Padgett 1996]) to allow placement of a bait station (see below).

At each station, a light meter will be used to determine the percentage of entrance light (Lux) reaching that station. These data will be collected only once, on a sunny, cloudless day. Light readings will be taken down to 1 Lux, but the twilight zone may include even dimmer light situations beyond the capacity of the meter. Therefore, when a secchi disk held at arms length by a dark-acclimated (30+ minutes in-cave dark) researcher is not visible, the station will be recorded as 0 Lux, while stations for which the disk is visible but there is less than one Lux will be recorded as <1 Lux.

Substrate at each marked station, and half way (2 m) between each station, will be characterized on a one-time basis using a plastic quadrat (0.1 m^2, [0.316 x 0.316 m]) placed in each of four positions: on the floor, each wall, and the ceiling (for vertical passage, the four major compass points - N, S, E, W - will be utilized). These substrate data will later be associated with bi-monthly cave invertebrate census data (see methods, below).

Data collected on a bi-monthly basis, In-cave sampling transect

Soil temperature (at 2 cm depth, less if impenetrable) will be measured at each marked transect station (at 4 m intervals) in the caves during each bimonthly visit.

Baited RIFA traps (identical to those described for surface bait sampling) will be placed at each (4 m interval) in-cave transect station for 20 minutes. Traps will be covered with cardboard squares to replicate the conditions of the surface timed bait traps (Figure 19). The traps will be placed and recovered immediately following surface bait trapping, or simultaneously if sufficient field crew is present. Data from these traps will be used to quantify the presence of foraging RIFA and document spatial and temporal patterns of cave utilization.

Cave fauna will be censused using the plastic quadrat (0.1 m^2) during each bi-monthly visit. The quadrat will be placed on the floor, each wall, and the ceiling (for vertical passage, the four major compass points - N, S, E, W - will be utilized) and the animals in each quadrat (including those observed moving out of the quadrat area as it is being placed) will be quickly tallied by most specific convenient taxon. This procedure will be repeated every two meters, utilizing the marked stations and unmarked midpoints between the stations. Anecdotal data on other invertebrates observed between quadrat stations may also be noted. The quadrat-based bimonthly cave fauna census will provide an estimate of in-cave density and distribution of cavernicoles that will be compared to in-cave bait trap data for RIFA.

Figure 19. Bait station in Shell Mountain Bat Cave, on bat guano, showing cardboard cover (future covers will be somewhat larger). Note small black guanophilic beetles (Histeridae). Photo by Steve Taylor, 8 August 2001.

Sequence of in-cave, bi-monthly sampling procedures

Because cave fauna, especially predators and cave crickets, tend to be secretive and move away from light (Campbell 1976) and human activity, the quadrat-based cave fauna census will be conducted prior to the in-cave RIFA bait trapping. Two people are needed to conduct the faunal census, thus, the logical procedure is as follows: 1. Starting at the entrance, one researcher with the 0.1 m^2 quadrat begins into the cave, calling out census data to the second person, who records those data; 2. Upon completing the last station (farthest into the cave), one of the researchers proceeds out of the cave, placing bait stations and measuring soil temperature at each station; 3. After 20 minutes, the second researcher comes out of the cave, collecting bait traps at the stations along the way.

Ceuthophilus secretus foraging range

Crickets (***Ceuthophilus secretus***) emerging from the cave to forage will be marked with a single color of fluorescent (UV bright) paint (Figures 20, 21) just outside the entrance. After painting, the crickets are released and generally continue out on their foraging excursions (personal observation, Ft. Hood, September 2001). The researchers then walk transects at regularly timed intervals (e.g., one hour intervals), searching for crickets (Figure 22) with a portable blacklight (UV light). Locations of crickets are flagged, and distances of flags from the cave entrance are measured the following day. Concerns include a) paint marks could interfere with cricket behavior; and b) searching

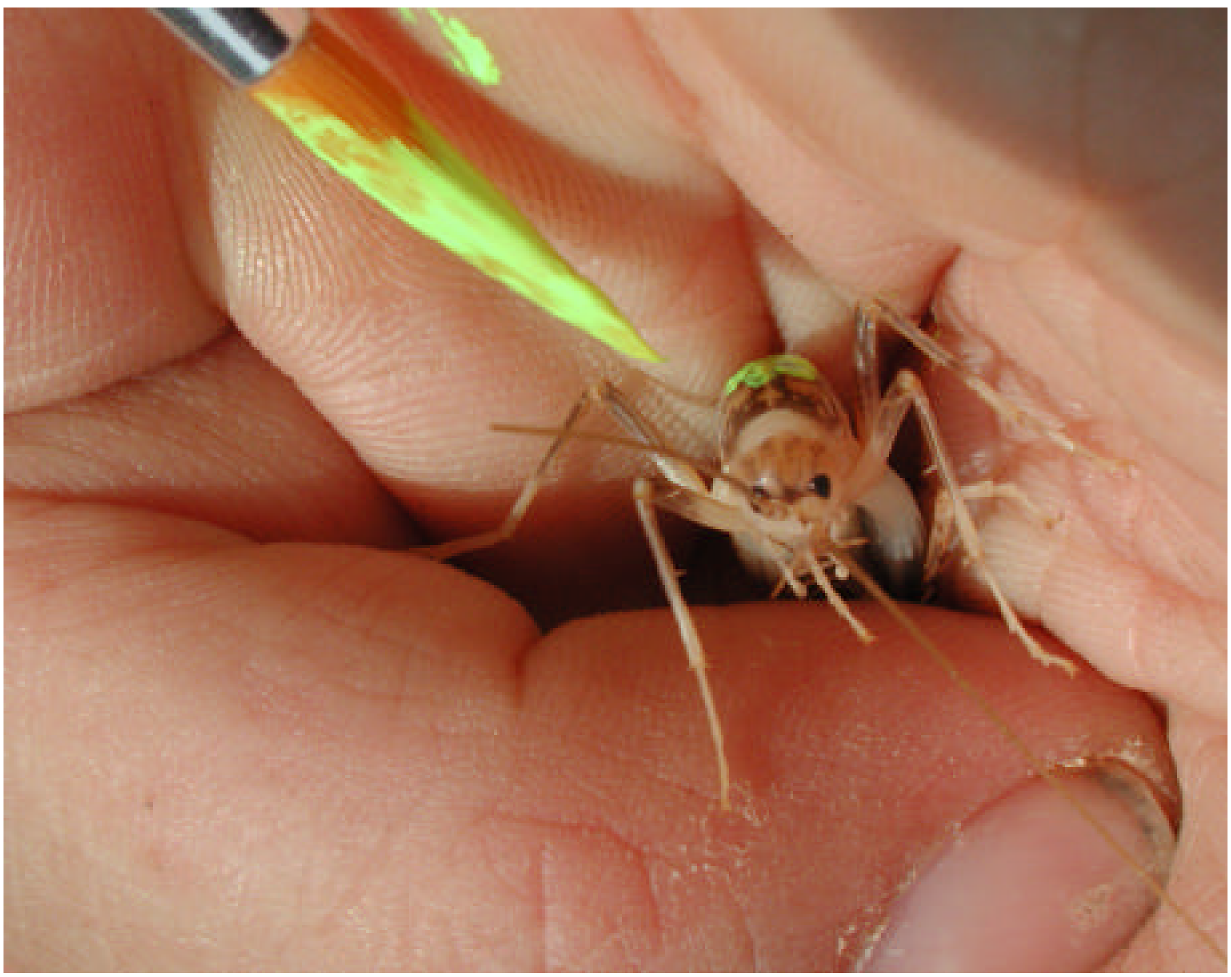

Figure 20. Painting the thorax of ***Ceuthophilus secretus*** with a fluorescent (UV bright) paint, Big Red Cave. Photo by Jean Krejca, 5 September 2001.

Figure 21. Marked ***Ceuthophilus secretus*** at entrance to Big Red Cave, ready to be released to forage. Photo by Steve Taylor, 5 September 2001.

Figure 22. Marked ***Ceuthophilus secretus*** foraging at night about 20 m from Big Red Cave. Yellow paint mark on cricket shows up very well under blacklight, but not in this flash-lit image. Photo by Steve Taylor, 5 September 2001.

for foraging crickets could alter their foraging behavior. While these concerns cannot be eliminated, preliminary trials did not demonstrate any obvious behavioral changes. This method has several advantages, a) only crickets emerging to forage are marked; b) many crickets, a high proportion of those emerging, can be marked; c) the caves are subjected to less disturbance; d) the effort expended to mark each cricket is considerably less than in-cave marking (for example, no need to use vertical caving techniques); e) large numbers of data points can be acquired; f) the emergence location of all foragers is known; g) lack of unnaturally rich bait stations makes it more likely that observed foraging distances are natural. Preliminary trials at Fort Hood (September 2001) demonstrated that this method should be quite effective.

RIFA impact on *Ceuthophilus secretus* foraging efficiency

RIFA/***Ceuthophilus*** interactions at bait stations will be examined by timing bait utilization at RIFA-excluded and RIFA-allowed bait stations. Design of the bait station is modified from Vinson (1991). Glass or glazed ceramic containers (high enough to exclude RIFA access, but low enough that the cave crickets can climb up to the top) are coated with Fluon® AD1[9] to prevent ants from climbing. The containers are placed on a square of cardboard and are covered with a similar square of cardboard serving as a platform upon which a small dish with bait (tuna) is placed (Figures 23-25). Bait stations will be set up only at selected caves and sampling will not be bi-monthly. Instead, baiting will take place at various times of year when both RIFA and ***Ceuthophilus secretus*** are actively foraging on the surface at night. Bait stations will be set up 2-20 m from a cave entrance. A single trial will consist of videotaping two adjacent (paired) bait stations (RIFA-excluded, RIFA-allowed) for a fixed time interval (perhaps 15 to 30 minutes, interval to be determined in preliminary trials). Video taping will commence immediately following bait station placement. Baits will be placed at night after crickets have begun to emerge from the cave. Several sequential taping sessions, with baits placed in different locations at least 5 m from other taping sessions, may be carried out on a single night at a single cave.

Figure 23. RIFA-allowed (left) and RIFA-excluded tuna bait stations. Cardboard platform is high enough off of the ground that RIFA cannot reach it without a ramp, and the dish beneath the cardboard is coated with a substance (Fluon®)[9] that the ants cannot climb. The cardboard platform is low enough that ***Ceuthophilus secretus*** can easily climb up to the bait. Adding cardboard strips leaning from the ground up onto the paper ramp allows access by RIFA for the RIFA-allowed bait stations. Note ***Solenopsis invicta*** foraging trails leading to RIFA-allowed station. Photo by Steve Taylor, 5 September 2001.

[9] Asahi Glass Fluoropolymers USA, Inc., PO Box 828519,Philadelphia PA 19182-8519.

Figure 24. ***Ceuthophilus secretus*** and ***Solenopsis invicata*** at RIFA-allowed bait station, feeding on tuna. Photo by Steve Taylor, 2 September 2001.

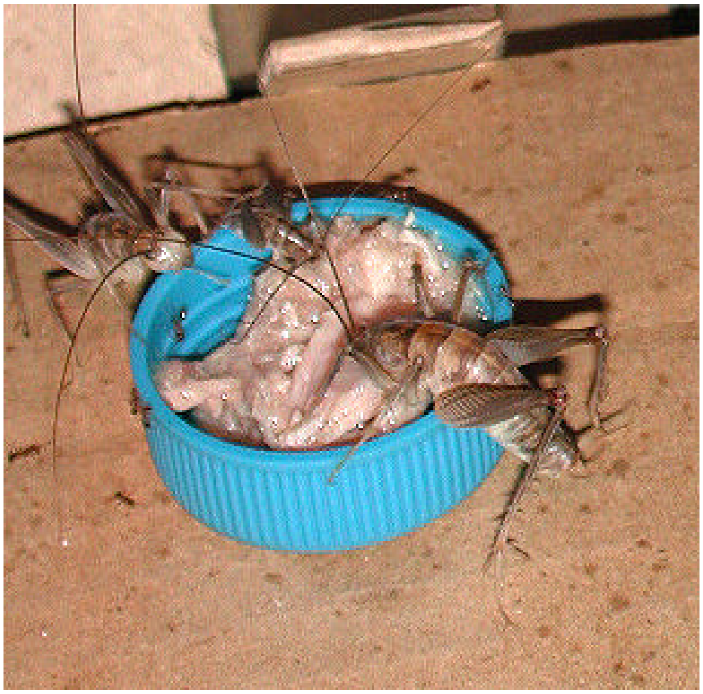

Figure 25. ***Ceuthophilus secretus*** and ***Solenopsis invicata*** at RIFA-allowed bait station, feeding on tuna. Photo by Steve Taylor, 2 September 2001.

For each bait station video, several factors may be quantified: time to first arrival of RIFA and ***Ceuthophilus***; duration of presence of individual ***Ceuthophilus*** on bait; and number of ants present on bait when ***Ceuthophilus*** arrives and leaves. Specific factors quantified may vary somewhat from the above; few preliminary trails could be completed prior to this report. At least 30 such RIFA-excluded + RIFA-allowed bait-pair videos will be filmed and analyzed. Based on observations made by Elliott (1992), I anticipate the sample protocol could provide insight into RIFA interference with ***Ceuthophilus secretus*** foraging. Preliminary field trials in September 2001 suggest that this method will work (see video clips on compact disk included with this report).

General Climatic data

At several (at least two) of the caves, temperature and relative humidity will be recorded using OnSet Hobo® data loggers bolted to the cave walls. These data will be collected on a regular basis throughout the year in the entrance and dark zones. In-cave humidity is often near 100% RH, and I am unaware of any data logger that accurately and reliably measures RH above 98%, so it is anticipated that the humidity data may be of little value.

At a location relatively central to the study are, epigean temperature and humidity will also be logged. Rainfall and other weather data may be collected from the nearest available weather station.

Major hypotheses

Some of the research questions posed above are purely descriptive (e.g., How far into caves do RIFA regularly forage?). Others invite the testing of specific hypotheses. Some such hypotheses are outlined below. Where significance is not defined below, $p \leq 0.05$ for appropriate statistical comparisons.

> H_0: There is not a significant presence of foraging RIFA in epigean habitats in the vicinity (30 x 30 m square plots) of important karst invertebrate caves (and,

therefore, we can assume the ants do not have a negative impact upon the epigean community or, through indirect effects, well-being of the cave community).

H_1: There is a significant presence of foraging RIFA, at least seasonally, in epigean habitats in the vicinity (30 x 30 m square plots) of important karst invertebrate caves (and, therefore, we can assume the ants have a negative impact upon the epigean community which is clearly important to the well-being of the cave community).

> 'Significant' here is defined as more than 16.67 % (1 out of 6) of the caves having more than 5 of 25 bait traps with 10 or more RIFA in at least one season of the year.

H_0: RIFA foraging in caves is not significant across all seasons.

H_1: RIFA foraging in caves is significant at at least one time of year.

> 'Not significant' will be defined, within each sample period (bi-monthly sampling) as no more than 16.67 % (1 out of 6) of the caves having 2 or more individuals of ***Solenopsis invicta*** in more than one bait trap, and no more than 16.67 % of the caves having more than 2 bait traps with RIFA present (excluding the trap at the entrance, which is not technically 'in' the cave). 'Significant' is any number and distribution of RIFA in bait traps exceeding the above.

H_0: RIFA foraging in caves (excluding entrance station) does not exhibit seasonal (bi-monthly sample period) trends.

H_1: RIFA foraging in caves (excluding entrance station) exhibits significant seasonal (bi-monthly sample period) differences.

H_0: Intensity of RIFA foraging in caves does not vary significantly among caves when the first four (five?) bait stations (starting from the entrance) are compared (all seasons pooled).

H_1: There are significant differences among caves in RIFA foraging intensity when the first four (five?) bait stations (starting from the entrance) are compared (all seasons pooled).

H_0: RIFA foraging activity in caves is random with respect to distance from the cave entrance.
H_1: RIFA foraging activity, as measured by bait traps, is significantly higher closer to the cave entrance.

Cave maps for the 15 caves from which study sites will be selected are found in Reddell (2001). These maps, and preliminary field reconnaissance, indicate that most of the caves have vertical entrances with a debris pile situated at the bottom of the entrance 'drop' or pit – typically in the twilight zone. Such accumulations of organic debris in caves commonly contain the richest and most diverse invertebrate fauna, as well as providing prime habitat for small vertebrates, such as salamanders. This energy rich source might be expected to attract foraging predators, such as fire ants. Preliminary field studies in August 2001 support this idea, which has also been mentioned by Elliott and Reddell (**CITATIONS??**). These observations suggest the following hypotheses:

H_0: Excluding the entrance bait station, the bait station(s) closest to the debris pile at the base of a cave entrance drop will not, on the average, have significantly more foraging RIFA than other bait stations closer to the cave entrance for those caves which have one or more bait stations between the entrance station and the station closest to the entrance drop debris pile.
H_1: Excluding the entrance bait station, the bait station(s) closest to the debris pile at the base of a cave entrance drop will, on the average, be characterized by the presence of significantly more foraging RIFA than other bait stations closer to the cave entrance for those caves which have one or more bait stations between the entrance station and the station closest to the entrance drop debris pile.

As discussed above, it seems likely that RIFA interfere with ***Ceuthophilus secretus*** foraging activity – either through competition for food resources or through predation upon the crickets – and in the proposed study, this is examined by filming RIFA-allowed and RIFA-excluded bait stations. The following hypotheses relate to this concept.

> H_0: There is no difference in ***Ceuthophilus secretus*** foraging at RIFA-allowed and RIFA-excluded bait stations, as measured by cricket-minutes spent at each kind of bait, by mean number of individual crickets that come to each bait.
> H_1: ***Ceuthophilus secretus*** foraging is significantly lower at RIFA-allowed bait stations than at RIFA-excluded bait stations, as measured by cricket-minutes spent at each kind of bait, by mean number of individual crickets that come to each bait.

> H_0: RIFA density (at RIFA-allowed bait stations) is not significantly correlated with ***Ceuthophilus secretus*** density at baits, as measured by estimated numbers of individuals of each species present at a bait.
> H_1: RIFA density (at RIFA-allowed bait stations) is significantly correlated with ***Ceuthophilus secretuss*** density at baits, as measured by estimated numbers of individuals of each species present at a bait.
>
> > This effect might be measured at the end of the sampling period or at several time intervals during the sampling period (video session of fixed length).

Minor hypotheses, not directly related to the research:

A number of questions, other than those that are the immediate focus of this study, could be asked of the data collected in the proposed study. Below are examples of several such questions, which might be investigated by examining data collected in the proposed study.

Porter and Tschinkel (1987) noted that larger workers may forage at higher temperatures because their surface area to mass relationship makes them more tolerant of both heat and desiccation (at least, below 36 ºC). Therefore, we can predict that foraging fire ants would tend to be larger in epigean samples than hypogean samples.

H_0: When epigean soil temperatures at 2cm are high (mean of 28-35 ºC), there is no significant difference in foraging worker size in epigean bait samples and hypogean bait samples (entrance bait station excluded).

H_1: When epigean soil temperatures at 2cm are high (mean of 28-35 ºC), there is a significant difference in foraging worker size in epigean bait samples and hypogean bait samples (entrance bait station excluded) (e.g., Figure 26).

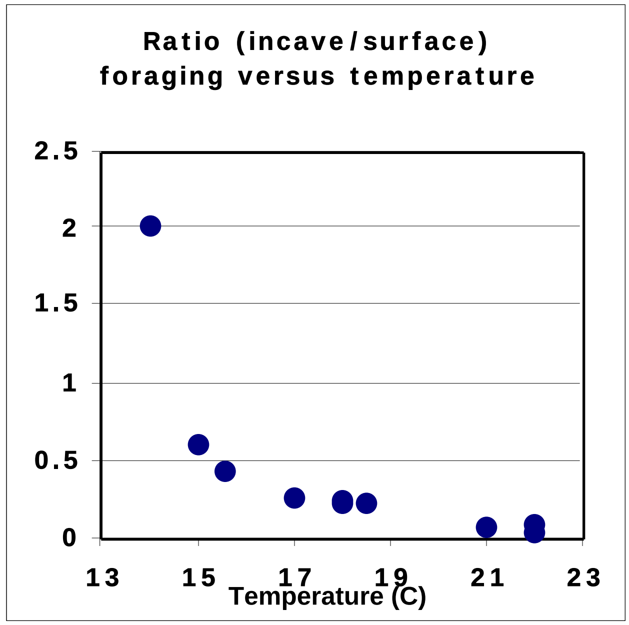

Figure 26. Hypothetical relationship between foraging ratio and epigean soil temperature at 2 cm depth.

When soil temperatures drop below 15 ºC, RIFA foraging ceases (Porter and Tschinkel 1987). Because cave temperatures in central Texas are moderated and well within the preferred range of ***Solenopsis invicta*** (Cokendolpher and Franke 1985), we might expect foraging activity to continue in caves after activity ceases above ground in the winter. This suggests a testable hypothesis.

H_0: As epigean 2 cm soil temperatures drop from 22 ºC toward 15 ºC and below, the value of the ratio of RIFA foraging intensity (as measured by bait trapping) in-cave (excluding entrance bait station) to the RIFA foraging above ground will display no significant pattern.

H_1: As epigean 2 cm soil temperatures drop from 22 ºC toward 15 ºC and below, the value of the ratio of RIFA foraging intensity (as measured by bait trapping) in-cave (excluding entrance bait station) to the RIFA foraging above ground will tend to increase.

Concluding Comments

The study design outlined above will provide quantitative baseline data on the extent of ***Solenopsis invicta*** impacts on cave invertebrate communities at Fort Hood. Information on the seasonal variations in impact, extent of impacts at varying distances into caves, potential for RIFA interference with cave cricket foraging, and quantitative information about the foraging range of cave crickets will be obtained. These data should prove helpful as resource managers attempt to protect Fort Hood's karst communities. The information also has broad applicability to cave resource management throughout central Texas and beyond. As a cautionary note, it is likely that some facets of the work outlined above will prove to be unfeasible or will be otherwise problematic, producing data of limited value – this is the nature of field work. Nonetheless, I am optimistic about the prospects for this study, and feel that significant contributions toward our understanding of RIFA impacts on karst invertebrate communities can be achieved.

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